

REVIEW REPORT

on Ph.D. thesis of Ing. Jan Fučík

Evaluation of the occurrence of selected pharmaceuticals in the soil ecosystem

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The submitted dissertation is quite extensive, 361 pages in total. From this number about 30 pages is theoretical part (Introduction, Current state, Analysis of pharmaceuticals). The largest part (Chapters 4-10, 252 pages) consists of transcriptions of already published publications, including supplementary information. The next Chapter 11 is a summary on 8 pages, and Chapter 12 is a list of references with 660 items on 46 pages. Chapter 13 is a list of 88 abbreviations used, Chapter 14 is author's CV, Chapter 15 and 16 contain list of author's publications.

The topic of the dissertation is undoubtedly highly topical in connection with the ever-increasing consumption of pharmaceuticals and their related presence in the environment, which has a number of negative impacts. The requirements of the circular economy are also quite significant.

When comparing the five scientific objectives of the dissertation defined on page 20 with the dissertation content, it can be stated:

1. The first goal - Development and optimisation of multiresidue analytical methods for pharmaceutical extraction from various matrices – was achieved. The method for determination of 42 drugs in soil, lettuce, and earthworms was developed and successfully published.
2. The second goal - Identification of pharmaceutical metabolites in *L. sativa* and *E. fetida* using LC-HRMS – was achieved. The method of metabolite identification based on the literature review, common metabolite pathways, and *in-silico* spectral library was successfully published.
3. The third goal - Fate of pharmaceutical residues in the environment: uptake by *L. sativa* in aquatic and soil systems and *E. fetida* in soil environment: I am not too happy with the wording of this goal's title. The uptake of drugs by earthworms and lettuce and the fate of pharmaceutical residues in the environment are quite different topics. But taking into

account only the second part of the title, I can conclude that this goal was also achieved and the results were published in 3 publications.

4. The fourth goal - Assessment of the ecotoxicological effect of pharmaceutical residues in the environment: fulfilled. The results of the ecotoxicological tests of the compounds under study can be found in four publications.
5. The fifth goal - Proposing solutions for addressing pharmaceutical residues in various environmental compartments: This goal was not fully achieved; from my point of view only experiments with advanced wastewater treatment methods can be mentioned here.

However, in the overall evaluation of the dissertation, it can be stated that the dissertation brought new knowledge regarding the fate of pharmaceutical residues in selected environmental components. The acquired knowledge was presented to the professional public in 7 publications in high-quality journals. Of these, the applicant is the first author in 4 publications and the corresponding author in the other two. The applicant's contribution to all the mentioned publications was significant. The solution used classical procedures and methods, as well as modified techniques (QuEChERS for the analysis of earthworms and lettuce) and non-traditional approaches (the application of the *in-silico* mass spectral library for the identification of drug metabolites). Therefore, the dissertation can be considered as an important contribution to the development of the scientific field.

The linguistic level of the submitted dissertation is very good; I have no critical comments in this area. Regarding the formal arrangement, I would criticise the applicant for not respecting the recommended ISO-690:2022 standard for the citation format. If he had respected this standard, the citation [40] would not have taken half a page. Also, the last citation [660] looks somewhat strange. Table 7.1 on page 174 looks a bit messy - some values are horizontally aligned to the centre, others to the top of the table cell.

I have the following comments regarding the content of the submitted dissertation:

1. On page 31 in chapter 3.1.2 Extraction techniques is stated, that: „...liquid-liquid extraction or Soxhlet extraction are very time-consuming and require significant amounts of laboratory glassware... [122]“. I strongly disagree. For liquid-liquid extraction, only separating funnel is needed (i.e. one piece of glassware), and the Soxhlet extractor consists of three glass parts. This is certainly not a significant amount of laboratory glassware, even if we take into account the additional containers needed for handling solvents and extracts. Furthermore, these techniques are extremely economical compared with other possibilities. By the way, I think that accelerated

solvent extraction (ASE) is the most popular modern method of extraction, not ultrasonic extraction (USE).

2. Comment to Table S5.1 (page 110) and other text: Atomic and molecular weights are not dimensionless numbers but are given in units **u** (unified atomic mass unit, $1 \text{ u} = 1.66054029 \times 10^{-27} \text{ kg}$, older version is **amu** – atomic mass unit) or **Da** (Dalton). The annotation states N.D. – not determined, but it is not used in this table at all.
3. Comment to Eqs. 7.1, 7.2 and 7.3 (page 172): I appreciate the use of time-weighted drug concentrations in soils to calculate the bioaccumulation factor in earthworms. Nevertheless, the above-mentioned equations are not placed in the optimal place in the explaining text. Moreover, Equation 7.3 could be solved analytically; the final shape should be presented.
4. Comment on Fig. 7.1. (page 177) and 7.2 (page 178): The concentrations of pharmaceuticals in the soil and earthworms on the y axes are given in $\text{ng} \cdot \text{ng}^{-1}$ – maximal values of $10\,000 \text{ ng} \cdot \text{ng}^{-1}$ in the soil are out of reality, as well as $3\,000 \text{ ng} \cdot \text{ng}^{-1}$ in earthworms. This error occurs both in the transcript of the publication in the dissertation and in the corresponding publication (Environmental Science and Pollution Research 31 (2024) 48351–48368, Fig. 1 on page 48358-48359).
5. In Table 8.1 on page 206 I was surprised by a single value in the Linearity range column - usually a range is characterised by two values - minimum and maximum.

I have the following questions regarding the content of the submitted dissertation:

1. In Table 7.1 on page 174 for clarithromycin in biochar amended soil is value for $k = 0.020831 \text{ day}^{-1}$, value of $\text{SE}(k) = 0.000024 \text{ day}^{-1}$ and coefficient of determination $R^2 = 1.0000$ – can you explain how you arrived at these results? The corresponding panel in Figure 7.1 on page 177 suggests that the situation might not be so straightforward.
2. On page 176 the effect of biochar on the rate of degradation of pharmaceuticals in soil is discussed. Do you have an idea about the mechanism of this effect? I would expect that biochar exhibits adsorptive but not catalytic effects.
3. Figure 7.3 on page 180 - effect of various concentrations of pharmaceuticals on the relative weight of the earthworms after 21 days: Do you have an explanation why the distribution of relative weight after 21 days at a concentration of 100 ng/g is much narrower than at the other concentrations and even than in the control?

4. Question to Figure 8.5 on page 218, panel 5B: The difference between control group and group of plants irrigated with water containing 50 µg/L of pharmaceuticals is marked as highly significant. But it doesn't look like that from the picture - can you comment?

In conclusion, I can state that the submitted dissertation fully meets the conditions set out in Section 47, paragraph 4 of Act 111/1998 Coll., on higher education institutions. Its author has sufficiently demonstrated creative abilities in the given field of research and the submitted dissertation fully meets the standard requirements for dissertations in the field of Chemistry and Technology of Environmental Protection.

Therefore, I confidently recommend the acceptance of the dissertation thesis submitted, and after its successful defence, I recommend that its author, Ing. Jan Fučík, will receive the academic title „philosophiae doctor“ - Ph.D.

Brno, April 16, 2025

prof. Ing. Josef Čáslavský, CSc.